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Genetic Recombination in Bacteriophage φX174

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Genetic recombination in bacteriophage φX174 usually takes place early in the infection process and involves two parental replicative form (double-stranded) DNA molecules. The host *rec*A protein is required; none of the nine known φX174 cistron products is essential. The products of a single recombination event are nonreciprocal and asymmetric. Typically, only one of the parental genotypes and one recombinant genotype are recovered from a single cell. An alternative, less efficient recombination mechanism which requires an active φX174 cistron A protein is observed in the absence of the host *rec*A gene product.

Genetic recombination is, for our purposes, an interaction between homologous double-stranded φX174 DNA molecules yielding a DNA molecule having a nucleotide sequence partly that of one parent molecule and partly that of the other (20). Previous genetic analysis in bacteriophage φX174 has demonstrated the occurrence of low recombination frequencies, high negative interference, and additivity of map distance between closely linked genetic markers (4, 5, 22, 23; C. Hutchison, Ph.D. thesis, California Institute of Technology, Pasadena, 1969; R. M. Benbow, Ph.D. thesis, California Institute of Technology, Pasadena, 1972). Recombinant formation in the closely related bacteriophage S13 has been examined extensively by the Tessman group (1, 2, 13, 26, 27-30, see Discussion section below) with similar conclusions.

We have investigated genetic recombination in bacteriophage φX174 by using two, three, and four factor genetic crosses (4) carried out in several different host strains under a variety of conditions. The effects, on φX174 recombination, of host genes and bacteriophage cistrons as well as various parameters such as allelic ratio, multiplicity of infection, and time of recombinant formation in the infection process, have been examined. The purpose of these experiments has been to outline the major pathway(s) of φX174 recombinant formation.

The yield of bacteriophage genotypes produced by individual recombination events (as opposed to the characterization of the behavior of bacteriophage populations) also has been studied. Boon and Zinder have reported (6-8) that the majority of individual recombination events in the (unrelated) filamentous bacteriophage f_1 generate one recombinant and one genotype identical to that of one of the parents. Our data suggest that this asymmetric, nonreciprocal recombination often occurs in bacteriophage φX174.

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MATERIALS AND METHODS

Bacterial strains. The strains of *Escherichia coli* used in these experiments, their relevant genotypes, and their typical uses are summarized in Table 1. Strains used in the physical characterization of φX174 recombination intermediates (3, 5, 31; R. Benbow, Ph.D. thesis, California Institute of Technology, Pasadena, 1972; R. M. Benbow et al., J. Mol. Biol., submitted for publication, 1973) are also included.

Bacteriophage φX17 mutants. Mutants of bacteriophage φX174 and preparation of the genetically pure stocks used in these studies were described previously (4, 5). The cistron to which each mutant is assigned is indicated in parentheses: for example, *am*3(E). Bacteriophage stocks were stored frozen (−20 C) in 0.05 M sodium tetraborate solution.

Media. KC broth, Denhardt starvation buffer, top agar, and bottom agar were described by Benbow et al. (4).

Genetic crosses. The host bacterial strain, most commonly HF4714 (the reference strain) or one of its isogenic derivatives, was grown in KC broth with aeration at 37 C to 10^8 cells/ml. The culture was made 0.003 M KCN and aerated for an additional 10 min.

A 0.5-ml amount of each of two freshly diluted bacteriophage stocks containing 2×10^8 PFU per ml in KC broth with 0.003 M KCN was mixed in a mating tube in an ice bath. A 0.2-ml amount (2×10^7 cells) of the KCN-treated bacterial culture was added to each mating tube.

After incubation at 37 C for 15 min, the mixture was diluted 1:100 into KC broth at room temperature. These cultures were vigorously aerated at 32 C

TABLE 1. *E. coli strains used to study φX174 recombinant formation*[a]

Strain	φX[s]	Thymine requirement	Recombination alleles	Suppressors	Relevant characteristics	Reference
HF4714	φX[s]	thy[+]	*rec*[+]	Su[+]$_{UAG}$[b]	(Reference strain) *arg*, *his*, *leu*, *thr*, *pro*	16
RMB120	φX[s]	thy[+]	*rec*[+]	Su[-][c]	Nonpermissive; isogenic to HF4714	
HF4712	φX[s]	thy[+]	*recA*	Su[+]$_{UAG}$[b]	*recA*-13; isogenic to HF4714	
RMB121	φX[s]	thy[+]	*recA*	Su[-][c]	*recA* nonpermissive; isogenic to HF4714	
Su2*am*	φX[s]	thy[-]	*rec*[+]	Su[+]$_{UAG}$[b]	Alternative wild-type, *thy*, *cyt*, *met*, *pro*, *his*	14
Su2*och*	φX[s]	thy[-]	*rec*[+]	Su[+]$_{UAA}$	*och* suppressor strain; isogenic to Su2*am*	14
CIT103	φX[s]	thy[+]	*rec*[+]	Su[+]$_{UAG}$	*op* suppressor strain, *lac*, *F*[-], T4[s], λ[s] Sm[r]	4
RMB101	φX[s]	thy[+]	*rec*[+]	Su[+]$_{UAG\text{ and }UGA}$	*am-op* double suppressor	4
C	φX[s]	thy[+]	*rec*[+]	Su[-]	Nonpermissive (reference strain) BTCC 122; grows in TPG	24
HF4701	φX[s]	thy[+]	*rec*[+]	Su[-]	*uvrA* nonpermissive; grows in TPG	16
HF4726[c]	φX[s]	thy[+]	*rec*[+]	Su[+]$_{UAG}$	Hfr donor of φX[s]	
HF4704[d]	φX[s]	thy[-]	*rec*[+]	Su[-][e]	*uvrA*, T$_1$[s]	18
C1704	φX[s]	thy[-]	*rec*[+]	Su[-][e]	*uvrA rep*3 transductant of HF4704	9
RMB110	φX[s]	thy[+]	*rec*[+]	Su[+]$_{UAG}$	*recB uvrA*	
H502[f]	φX[s]	thy[-]	*rec*[+]	Su[-][e]	*endoI uvrA*, descendant of HF4704	31
H560[f]	φX[s]	thy[-]	*rec*[+]	Su[-][e]	*PolA*, *endoI*, (*PolA* from Cairns' mutant, other markers from H502)	
1000[f]	φX[s]	thy[-]	*rec*[+]	Su[-][e]	*PolA*, Sm[r], F[+], *endoI* (*PolA* from Cairns' mutant, other markers from H502)	
HF4705[d]	φX[s]	thy[-]	*rec*[+]	Su[-]	*uvrB*-5, T$_1$[s], *PurI*, *gal*, *arg*, *his*, T$_6$[s], Sm[r]	
HF4705[d]	φX[s]	thy[-]	*rec*[+]	Su[-]	*uvrB*-5, T$_1$[s], *PurI*, *gal*, *arg*, *his*, T$_6$[s], Sm[r]	
HF4706[d]	φX[s]	thy[-]	*rec*[+]	Su[-]	*uvrC*-34, T$_1$[s], *PurI*, *gal*, *arg*, *his*, T$_6$[s], Sm[r]	
*ts*7	φX[s]	thy[-]	*rec*[+]	Su[-]	Temperature-sensitive DNA ligase; *arg*, *met*, *pro*, *ura*, *trp*; descendant of TAU-bar	21
K12(W6)	φX[R]	thy[+]	*rec*[+]		Spheroplast assay	25
K12-NH4547[g]	φX[R]	thy[-]	*recA recB uvrA*			
K12-NH4545	φX[R]	thy[-]	*recA recB*	*Su*[+]$_{UAG}$	Spheroplast assay, *recA recB*, *trm*, *his*, *pro*, *leu*, *arg*, *gal*, *lac*, *mtl*, *nyl*, *ara*, str R	

[a] The strains in this table are stored frozen (−50 C) in the R. L. Sinsheimer collection. Inocula are obtained by growing up the frozen stock overnight at 37 C in KC broth.

[b] This strain suppresses most *am* mutations, but is unable to plate *am*80(H), *am*90(H), or *am*89(F) efficiently.

[c] Selected by a technique analogous to that of Baker et al. (1).

[d] Constructed in this laboratory by Paul Howard-Flanders; made *thy*[-] by T. Komano.

[e] This strain is more stringently nonpermissive than C for the *och* mutants used in these studies.

[f] A gift from Hoffman-Berling.

[g] A gift from Paul Howard-Flanders.

for 90 min, diluted in Denhardt starvation buffer, and titered immediately. To obtain maximum reproducibility, these procedures should be followed exactly; the addition of chloroform, or the use of other dilution buffers, and other seemingly insignificant variations often resulted in at least twofold fluctuations in observed recombination frequencies.

Measurement of wt recombinants. Three parameters have been variously used to describe the amount of φX174 recombinant formation in two-factor genetic crosses: recombination frequency, wild-type (*wt*) recombinants per infected cell, and recombinant infective centers.

The recombination frequency is defined as the number of *wt* recombinants divided by the total number of progeny phage. Specific definitions of the recombination frequency for each two-, three-, or four-factor cross in terms of plating strains and incubation temperatures are given by Benbow et al. (4).

The number of *wt* recombinants per infected cell is used more frequently in this paper to minimize the effects of nonequivalent physiological conditions with different host strains or among different bacteriophage mutants. For example, the number of *wt* recombinants formed per cell in a genetic cross carried out under permissive and nonpermissive growth conditions is usually similar, although the recombination frequencies are very different because of the small number of progeny bacteriophage produced under

nonpermissive conditions.

The proportion of cells in which *wt* recombinants are formed (the infective centers assayed on nonpermissive host strains) was often measured in order to confirm results obtained by either of the previous methods.

Control "selfings" were carried out for all the mutants used in the crosses described in this study. The recombination frequency for each mutant, mated with itself, was always below 5×10^{-6} *wt* per progeny phage; the number of *wt* recombinants (revertants) per cell did not exceed 5×10^{-4}; the number of revertant infective centers per cell was less than 5×10^{-5}. Experiments in which control selfings exceeded these values were discarded. Thus, the recombination data given in this paper usually are at least two orders of magnitude above these background measurements.

Three plates were counted for each dilution; two (or more) dilutions were used to estimate the number of *wt* recombinants per milliliter. Cell concentrations were estimated by counting about 100 cells in a Petroff Hausser bacteria counter; the error in this number is roughly ±10%. To calculate the means and standard deviations in Tables 2 and 3, the values for the titer of the phage were divided by the concentration of cells (usually 1.67×10^5 cells/ml) without correction for the ±10% uncertainty in cell count. No correction was made for inviability, although viable cell titers were often verified by plating the cells prior to KCN treatment. Since the cell concentrations estimated by the two methods were identical within 20%, the Petroff Hausser count was employed. Cell doublets were counted as one cell if they were not separated by gentle vortexing.

Control experiments involving the temperature-sensitive host cell *ts*7 were carried out entirely at 30 C.

TABLE 2. *Effect of host genes on φX174 recombinant formation in the cross am10(D) × am9(G)*

Host strain	Relevant genotype	φX174 *wt* per cell ($\times 10^2$)	φX174 recombinants/cell (normalized to *wt* host)
HF4714	*wt*	5.0 ± 2.1	1.00
HF4712	*recA*	0.7 ± 0.3	0.14
RMB110	*recB*	6.4 ± 1.1	1.28
HF4704	*uvrA*	8.5 ± 1.3	1.7
*ts*7	*lig*	4.6 ± 0.8	0.92
1000	*polA*	4.8 ± 0.9	0.96
NH4545[a]	*recA recB*	0.12 ± 0.04	0.025
NH4547[a]	*recA recB uvrA*	0.09 ± 0.03	0.018
$Su2_{am}$	*wt*	3.4 ± 0.8	0.68
$Su2_{och}$	*wt*	4.0 ± 0.8	0.80
H502	*uvrA endoI*	3.1 ± 1.0	0.62
HF4705	*uvrB*	7.6 ± 1.1	1.52
HF4706	*uvrC*	6.9 ± 1.3	1.38
C	Pm⁻	8.3 ± 1.1	1.66

[a] Spheroplast assays were used (R. M. Benbow, Ph.D. thesis) since these strains are ϕX^R.

MOI and allelic ratios. The effect of MOI (input of PFU per cell) on recombinant formation, measured by the criterion of *wt* recombinants produced per cell, was examined for the following two-factor genetic crosses: *am*N1(H) × *am*87(F) and *am*3(E) × *am*9(G). The standard procedure for genetic crosses (see above) was employed. The total number of input phage was varied while the ratio of infecting genotypes (the allelic ratio) was kept constant at 1:1.

The allelic ratio for the genetic crosses listed above was also varied while the total MOI was kept constant at 20 phage per cell. This higher total MOI was selected because it permitted the use of a wider range of allelic ratios while reducing the effect of changing the MOI of each individual genotype.

The results are presented for the two-factor cross *am*87(F) × *am*N1(H). Similar data for the two factor cross *am*3(E) × *am*9(G) are presented by R. M. Benbow (Ph.D. thesis, California Institute of Technology, Pasadena, 1972).

Effect of φX174 cistrons. The indicated two-factor crosses were carried out in four isogenic host strains: HF4714; H4714 su⁻ (RMB120); HF4712; and HF4712 su⁻ (RMB121). The number of *wt* recombinants per cell, defined as the titer on *E. coli* C divided by the total number of infected cells, was used to determine if any viral cistron product is essential for φX174 recombinant formation.

φX174 genetic recombinants in bursts of single cells. *E. coli* C, a nonpermissive host for the *am* and *op* mutations used in these experiments, was grown to 10^8 cells per ml in 10 ml of KC broth at 32 C with aeration. KCN solution was added to give a final concentration of 0.003 M, and the cells were aerated at 37 C for 10 min. A 1.0-ml amount of this suspension was added to 2.5 ml of *am*88(F) *ts*79(G) and 2.5 ml of *op*6(F), each at 2×10^8 PFU per ml in KC broth, 0.003 M KCN. The mixture was incubated at 32 C for 20 min, centrifuged in a Sorvall SS34 rotor at 7,000 rpm for 10 min, and the pellet was washed three times in 6 ml of cold KC broth to remove unadsorbed phage. A 0.2-ml amount of the pellet resuspended in 6 ml of KC broth was removed and used as a control to measure the recombination frequency under standard conditions.

The remaining mating mixture was diluted in cold KC broth to 1.67 cells/ml, based on the presumed starting concentration of 1.67×10^7 cells/ml. (The actual concentration of cells is unimportant as long as there is no more than one burst for every 10 tubes.) Five hundred 1.0-ml portions were incubated at 32 C for 2 h, and then were plated on the assay system described below.

The genotypes of the progeny from this cross were determined by a double-layer method. Plates were first layered with 2.5 ml of top agar containing the nonpermissive host strain *E. coli* C. Then the single-burst tubes were plated over this layer with an additional 2.5 ml of top agar containing RMB101, an *am op* double suppressor. Incubation was at 30 C for 4 h, and then at 40 C overnight. Tentative classification was made as follows: *wt* recombinants generated clear large plaques; *op*6(F) parental mutants gave medium sized turbid plaques; *am*88(F)*ts*79(G) parental mutants produced very small turbid plaques; control

TABLE 3. *Effect of φX174 cistrons on φX174 recombinant formation in a wt host*

Cistron	Cross	Wt per cell × 10^2		Fraction of Su⁺ *wt* recombinants made in Su⁻ cells
		(HF4714) Su⁺	(RMB120) Su⁻	
A	*am*18(A) × *am*86(A)	7.6 ± 1.8	18.8 ± 3.7	2.5
A	*am*33(A) × *am*50(A)	4.1 ± 1.4	11.3 ± 2.8	2.8
B	*am*14(B) × *am*16(B)	1.8 ± 0.3	1.5 ± 0.4	0.8
D	*am*10(D) × *am*H81(D)	0.9 ± 0.3	1.3 ± 0.1	1.4
E	*am*3(E) × *am*27(E)	0.6 ± 0.3	0.2 ± 0.1	0.33
F	*am*88(F) × *op*6(F)	13.5 ± 1.2	16.8 ± 1.9	1.2
G	*am*9(G) × *am*32(G)	1.3 ± 0.2	0.9 ± 0.3	0.7
H	*am*N1(H) × *am*80(H)	0.4 ± 0.2	0.5 ± 0.1	1.3

experiments showed that *op*6(F)*am*88(F) double mutants also give small turbid plaques which are easily resolved from the *am*88(F)*ts*79(G) parents by plating on HF4714 (*am* suppressor only).

The tentatively identified plaques were then picked with a sterile glass tube, resuspended, and assayed on the appropriate suppressor stains. We gratefully acknowledge the help of Jill Fabricant Hiatt with these assays, which were particularly difficult due to the necessity of constructing and using φ80 resistant strains (since the *am-op* double suppressor is lysogenic for φ80).

A similar, but more easily analyzed, single-burst assay system was subsequently developed for the cross between the mutants *am*10(D) and *am*80(H). The results obtained with this system will be described elsewhere.

RESULTS

Genetic map of bacteriophage φX174. The order of φX174 cistrons was established by the three factor genetic crosses described by Benbow et al. (4; see also 2). Cistron boundaries were drawn by using the molecular weight of the protein assigned to each cistron (5) after accepting the suggestion of Linney et al. (19) that cistron A codes for a 67,000-molecular-weight polypeptide, and that the 34,000-molecular-weight polypeptide previously assigned to cistron I represents a fragment of the cistron A product (19).

The net effect of Linney's suggestion (19) on the assignments in Table 1 of Benbow et al. (5) is as follows. Cistron A codes for a protein of 67,000 molecular weight; the other cistron product of 14,000 molecular weight decreased by cistron A mutants is not identified with any cistron at present. The 34,000-molecular-weight protein which was (potentially) cistron I is now a cistron A polypeptide fragment; however, since cistron I's existence (17) is now retracted by Hayashi, we have lost one gene and one protein. By elimination, this implies that cistron C must code for the 7,000-molecular-weight protein. The other seven cistron products are those previously identified. Note also that the change proposed by Linney does not change the proportions of the genetic map except in the cistron A and C regions. Since cistron C contains only one genetic marker, the suggestion of genetic and physical proportionality (5) is unchanged by this alteration.

Cistron I, originally proposed by Hayashi and Hayashi (17), is no longer believed to exist (M. Hayashi, personal communication). The genetic map thus derived is shown in Fig. 1 and is included to help characterize the crosses below and to compare with the genetic map in *rec*A host. It is nearly identical to that given earlier (4), but now has defined cistron boundaries and is proportional to physical distances on the φX174 genome. The putative function of each cistron product is indicated.

MOI and allelic ratios. The genetic crosses employed during our studies of φX174 genetic recombination (3–5, 31; R. M. Benbow et al., J. Mol. Biol., submitted for publication, 1973) were all carried out at a standard MOI of 5 PFU of each of the two infecting genotypes (i.e., total MOI = 10; allelic ratio 1:1). The effect of varying the total MOI or the allelic ratio on recombinant formation is shown in Fig. 2.

Wt recombinant formation is relatively independent of total MOI in the range of 2 to 20 (Fig. 2a). Similarly, the allelic ratio may be varied from 1:6 to 6:1 without major effect (Fig. 2b). These conclusions also apply to the recombination frequencies since the burst size is independent of either MOI or allelic ratio over an even larger range (compare 27). It should be noted, however, that we did not attempt to correct for any additional input of phage DNA due to infection by defective (non-plaque-forming) phage particles.

Effect of host alleles. The two-factor genetic cross, *am*10(D) × *am*9(G), was carried out in a variety of host strains with the results shown in Table 2. The effects demonstrated were confirmed by the crosses *am*N1(H) × *am*87(F),

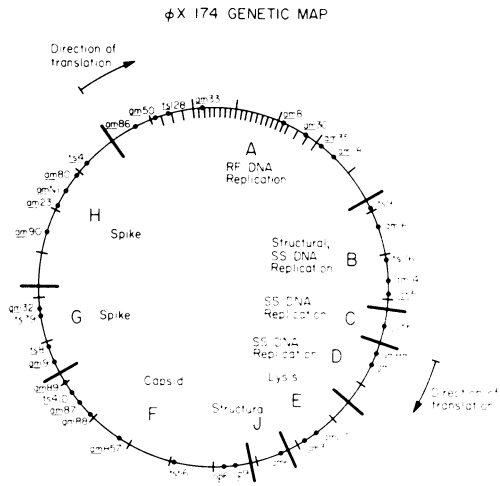

FIG. 1. *Frequency of wt recombinants in two factor genetic crosses between φX174 conditional lethal mutants is represented schematically. One map unit represents 10^{-4} wt recombinants per total progeny phage. Within cistron A the hatch marks on the outer circumference represent the recombination frequencies "across" cistron A (see 4); those on the inner circumference represent intracistronic recombination frequencies. Two distinct translational origins (at the beginnings of cistron D and A) are believed to exist (5). Cistron boundaries are drawn in accordance with the molecular weights of the cistron products characterized in (5) and (19). Cistron A recombination frequencies are not proportional to physical distance for reasons discussed elsewhere (4, 5; R. M. Benbow et al., J. Mol. Biol., submitted for publication, 1973). Putative cistron functions are included for orientation.*

*am*3(E) × *am*9(G), and *am*9(G) × *am*N1(H) (data not shown). The host *rec*A mutation reduces φX174 genetic recombination 3- to 100-fold dependent on the markers used (27) (see below). The host *rec*B mutation alone has no significant effect; in a double mutant with *rec*A or with *rec*A *uvr*A it effects a further 5- to 10-fold reduction in *wt* recombinant formation. The other host alleles tested, either singly or in combination, had little or no effect. The *sbc*A and *sbc*B alleles (which suppress the *rec*B phenotype) have been shown by Alvin J. Clark to have little effect on φX174 genetic recombination either singly or in combination with other markers (personal communication).

Effect of φX174 cistrons. In *wt* cells, the products of the known φX174 cistrons do not appear to be required for most *wt* recombinant formation. This is shown in Table 3 by the fact that the number of *wt* recombinants per cell in intracistronic crosses is similar (within a factor of three) in permissive and in nonpermissive hosts (the cistron product is not made in the nonpermissive host). This conclusion was confirmed by measuring the number of cells that form *wt* recombinants (data not shown, see 1); this verification by measuring infective centers minimizes the possibility that the data in Table 3 are significantly altered by selective growth of *wt* recombinants in nonpermissive cells. The dispensability of all φX174 coded functions suggests that the major pathway for *wt* recom-

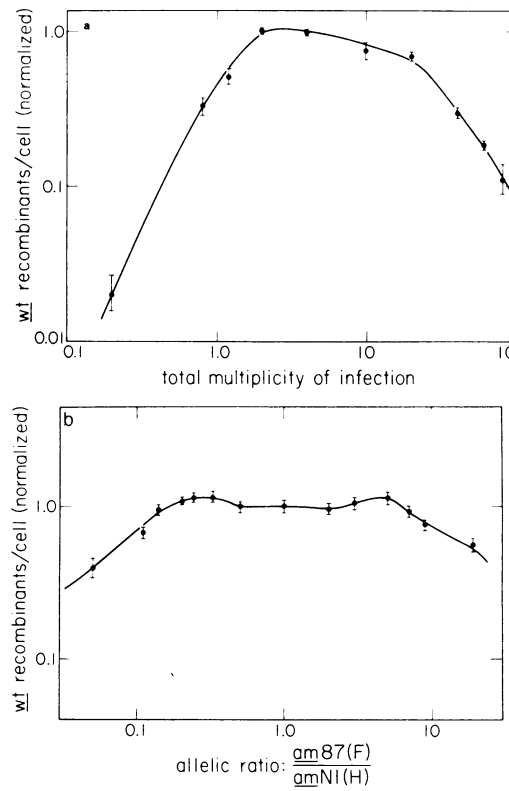

FIG. 2. *φX174 wt recombinant formation as a function of total multiplicity of infection or of allelic ratio. The highest yield of wt recombinants in the MOI experiment was arbitrarily set equal to 1.0. This optimal recombination corresponded to 4.25×10^{-2} wt recombinants per cell (average burst size: 132 ± 21 phage/cell) for the cross amN1(H) × am87(F). The error bars indicate standard deviations calculated as in (4). (a) Wt recombinants per cell are plotted versus the total multiplicity of infection [amN1(H) + am87(F)]. (b) Wt recombinants per cell are plotted versus the allelic ratio [am87(F)/amN1(H)] at a total MOI of 20. The two factor crosses were carried out in the host strain HF4714 as described in Materials and Methods. Total burst sizes were unaffected by different allelic ratios, but were significantly decreased (<50%) at MOI above 40 or below 1.0. The yield of wt recombinants/cell at an allelic ratio of 1:1 was set equal to 1.0. This corresponded to 3.9×10^{-2} wt recombinants per cell (average burst size 171 ± 18).*

binant formation shares many features with the *E. coli recA* pathway (10) rather than representing a φX174 specific mechanism.

Since the host *recA* gene product is required for the major pathway of recombinant formation, it is of interest to determine if the lack of any φX174 cistron affects recombination in the absence of the host *recA* gene product (in analogy to the *red* genes of bacteriophage λ). These recombinants must be formed by an alternate (non-*recA*) mechanism. As can be seen in Table 4, lack of the φX174 cistron A product further markedly reduces φX174 recombinant formation in a *recA* host. This effect, which is also observed in the related bacteriophage S13 (1), may reflect a requirement for cistron A product in φX174 progeny RF DNA replication rather than for recombinant formation per se. No other φX174 cistron product is required for recombinant formation in a *recA* host.

It should be emphasized, however, that the major pathway involving the *recA* protein is independent of newly synthesized cistron A product. (Indeed, as observed in Table 2, the major pathway may be slightly inhibited by the gene A product.) Nevertheless, it should also be noted that we cannot exclude the presence and possible role of small amounts of active φX174 cistron products carried in by the infecting genome.

Genetic map in a recA host. The host *recA* allele is the only host gene of those tested which is implicated in the major pathway of φX174 recombinant formation. It was of interest, therefore, to determine the genetic map of bacteriophage φX174 in a *recA* host. Such a map would represent the *wt* recombinants formed by other mechanisms (for example, Tessman's secondary mechanism (27, 28)). A comparison of the φX174 genetic map in a *recA* host with that formed in a *wt* host may indicate regions of the genome (or specific phage mutants) which preferentially recombine under the influence of the *recA* protein. In addition, we wish to document very carefully the reduction of recombination frequencies by the host *recA* mutation with a large variety of φX174 mutants.

The φX174 genetic map in strain HF4712, a *recA* host, is shown in Fig. 3a. One map unit represents 10^{-4} *wt* recombinants per total progeny phage. As expected, the genetic map is circular, with cistron order DEJFGHABC; high negative interference is far lower than observed in crosses in a *wt* host. More details are yet to be published by R. Benbow and R. Sinsheimer. The length of the genetic map is $6 \pm 2 \times 10^{-4}$ *wt* recombinants per total progeny phage. The *recA* genetic map and the physical map based on polypeptide molecular weights (5) are approximately proportional over the entire genome, including the cistron A region. In addition, since high negative interference is considerably lower in the *recA* host, recombination frequencies can be read directly off this map (compare with Table 5) for most markers ($\pm 1 \times 10^{-4}$ *wt* per total progeny phage).

For comparison, the genetic map of Benbow et al. (uncorrected for the physical size of cistrons) (4) is reproduced in Fig. 3b. It is notable that the high recombination region within cistron A is considerably reduced in the *recA* host strain. This is further documented in Table 5. This implies that more genetic exchanges are observed with cistron A in a *wt* host than is expected from the physical size of the region. A possible explanation for this observation, proposed by Benbow et al. (J. Mol. Biol., submitted for publication, 1973), (which cannot be attributed to the cistron A product) (15) suggests that the region contains specific single-strand breaks which promote genetic exchange mediated by the host *recA* gene product. This hypothesis accounts for the fact that cistron A markers within the high recombination region

TABLE 4. *Effect of φX174 cistrons on φX174 recombinant formation in a recA host*

Cistron	Cross	Wt per cell × 10^2 *recA*		Fraction of Su⁺ *wt* recombinants made in Su⁻ cells
		(HF4712) Su⁺	(RMB121) Su⁻	
A	*am*18(A) × *am*86(A)	0.08 ± 0.03	0.003 ± 0.001	0.04
A	*am*33(A) × *am*50(A)	0.05 ± 0.02	0.006 ± 0.003	0.12
B	*am*14(B) × *am*16(B)	0.21 ± 0.07	0.33 ± 0.05	1.6
D	*am*10(D) × *am*H81(D)	0.10 ± 0.05	0.15 ± 0.03	1.5
E	*am*3(E) × *am*27(E)	0.07 ± 0.01	0.02 ± 0.01	0.3
F	*am*88(F) × *op*6(F)	1.75 ± 0.40	2.05 ± 0.40	1.2
G	*am*9(G) × *am*32(G)	0.30 ± 0.05	0.21 ± 0.04	0.7
H	*am*N1(H) × *am*80(H)	0.07 ± 0.01	0.10 ± 0.03	1.4

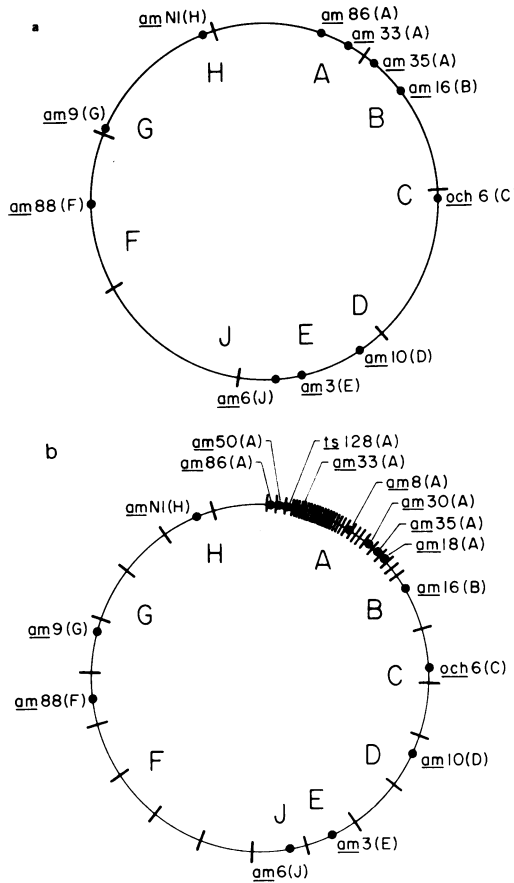

FIG. 3. *(a) The frequencies of wt recombinants in two-factor genetic crosses between φX174 conditional lethal mutants in a recA host (HF4712) are represented schematically. One map unit (the distance between two bars) represents 10^{-4} wt recombinants per total progeny phage. The specific two- and three-factor crosses employed are given in Table 4 in R. M. Benbow's Ph.D. thesis, and in (4). The error in the position of any single marker or in the total genetic length is less than $\pm 1 \times 10^{-4}$ wt recombinants, based on standard deviations calculated as in (4). (b) Frequency of wt recombinants in two-factor genetic crosses between φX174 conditional lethal nonsense mutants in a wt host (HF4714) (4), for comparison. One map unit also represents 10^{-4} wt recombinants per total progeny phage, so this figure may be compared directly with that in (a). The assumption, described in (4), that formal recombination events within the cistron A "loop" usually occur in pairs is invoked to explain the relative inertness of the cistron A region in recombination events with the rest of the genome.*

behave differently than cistron A markers outside that region (Table 5, see also R. M. Benbow et al., J. Mol. Biol., submitted for publication, 1973).

Single burst of φX174 recombinants. In the cross *am*88(F)*ts*79(G) × *op*6(F), *wt* recombinants are generated with comparable efficiency in either permissive or nonpermissive host cells (Table 6). By carrying out this cross in the nonpermissive host, single-burst experiments were feasible since, in theory, only those cells in which a *wt* recombinant is generated will produce a burst of phage. This is supported by the fact that only 41 of 500 tubes produced any plaques by using our assay system, even though

TABLE 5. *Effect of the host recA gene on φX174 recombinant formation*

Cross	HF4714 *rec*+[a]	HF4712 *recA*[a]	Ratio φX174 *wt* made in Rec+/ RecA
*am*3(E) × *am*9(G)	6.8 ± 0.8	2.3 ± 0.7	2.9
*am*N1(H) × *am*9(G)	3.1 ± 0.8	0.7 ± 0.2	4.5
*am*9(G) × *am*88(F)	1.3 ± 0.2	0.47 ± 0.2	2.8
*am*9(G) × *am*10(D)	5.9 ± 2.1	1.7 ± 0.8	3.4
*am*N1(H) × *am*10(D)	4.6 ± 0.3	2.05 ± 0.7	2.2
*am*N1(H) × *am*86(A)	2.1 ± 0.3	0.8 ± 0.4	2.6
*am*N1(H) × *am*33(A)	7.5 ± 1.2	0.6 ± 0.3	12.5
*am*18(A) × *am*86(A)	11.7 ± 2.4	0.8 ± 0.2	14.6
*am*18(A) × *am*33(A)	21.9 ± 3.2	0.3 ± 0.1	73.0
*am*33(A) × *am*35(A)	21.2 ± 2.4	0.2 ± 0.1	106.0

[a] In *wt*/total progeny phage × 10^4.

TABLE 6. *Single burst of φX174 recombinants*

Cross	Host	*wt*/ Burst
*am*88(F) *ts*79(G) × *op*6(F)	Su+ (RMB101)	18.5
*am*88(F) *ts*79(G) × *am*88(F) *ts*79(G)	Su+ (RMB101)	0.13
*op*6(F) × *op*6(F)	Su+ (RMB101)	0.09
*am*88(F) *ts*79(G) × *op*6(F)	Su− (C)	26.6

Single bursts[a]	*am*[b]	*op*	+	*am-op*
1	47	3	51	0
2	62	5	19	0
3	2	97	31	0
4	0	35	31	0
5	34	1	39	0
6	87	2	4	0
7	67	3	31	0
8	1	94	13	0
9	0	85	21	0

[a] The nine single bursts examined took place in nonpermissive (Su−) host cells; thus, only those crosses which yielded *some wt* recombinant phage were observed.

[b] The segregation of the *ts*79(G) marker was not followed in all of the *am* phage found in these bursts.

each tube contained an average of 1.67 infected cells. It should be noted that these single-burst experiments are subject to the criticism that there is selection for a particular class of recombinants (the *wt* recombinant) and to the objection that we will detect the progeny of only those cells in which the recombinant *wt* genome is subsequently transcribed and translated.

The genotype of each of the plaques found in the nine single bursts, which when plated produced more than three *wt* plaques, is presented in Table 6. The smallest of these nine bursts produced 66 PFU. (The remaining 32 single bursts produced one, two, or three *wt* plaques when plated; these were screened [see Materials and Methods] and were found to contain both parents in somewhat unequal ratios [usually less than 2:1]; thus the *wt* plaques found in these small bursts may represent revertants generated during infection [12].)

Each of the nine single bursts yielded phage of predominantly one parental genotype and one recombinant genotype. In our experiment, only the *wt* recombinant is selected; however, the reciprocal recombinant (the *am*88(F)*op*6(F) double mutant) would be detected if it were generated in the same cell as the *wt*.

Control selfings [*am*88(F)*ts*79(G) × *am*88(F)ts79(G) and *op*6(F) × *op*6(F)] yielded no single bursts producing more than three *wt* plaques. The first control cross had 18 small *wt* bursts out of 500 tubes, the second 47 out of 500. (A few [15] of these bursts did not contain *wt* plaques. We assume this is because the *wt* revertant, after being generated and transcribed, was not replicated in time to be included in the packaged phage particles.) No large bursts of *wt* (>3 phage/cell) were observed in these "selfings." Thus it is likely that the large bursts we have examined are, in fact, the results of recombination, whereas some or most of the small bursts represent revertants generated during infection. (The small fraction of observed single bursts [9/500 tubes] suggests that none of the large bursts contain more than one recombination event.)

DISCUSSION

The purpose of this paper is to outline the major pathway for φX174 recombinant formation. Several of the conclusions reached are in accord with those suggested for bacteriophage S13 by the Tessman group (1, 27) and for bacteriophage f_1 by Boon and Zinder (8). In addition, an alternative, independent mechanism for generating φX174 recombinants in the absence of the host *rec*A gene product is partially characterized.

Recombination usually occurs between two parental, replicative-form DNA molecules early in the infection process. φX174 cistron A mutants form *wt* recombinants at nearly normal frequencies in *wt* hosts without the synthesis of functional cistron A product (Table 3) and in the absence of progeny RF DNA molecules (1). Similarly, *wt* recombinants are formed at normal frequencies in *rep*3 cells (in which only the parental RF is formed (11)) (R. M. Benbow, Ph.D. thesis, California Institute of Technology, Pasadena, 1972). Finally, *wt* recombinants are detected during density exchange experiments in regions of CsCl equilibrium density gradients containing only parental DNA (R. M. Benbow, Ph.D. thesis, California Institute of Technology, Pasadena, 1972). Thus, we conclude that φX174 recombinants can be formed from parental RF DNA molecules.

Large single bursts of *wt* recombinants were observed (Table 6) under conditions where the average number of *wt* recombinants per cell was normal. (Similar conclusions could be drawn by reinterpreting the quasi-single-burst experiments of Denhardt and Silver [12].) This suggests that φX174 *wt* recombinants can be formed early in infection.

The evidence that φX174 recombination usually occurs early in the infection between parental RF DNA molecules is indirect. Spheroplast assays of RF DNA extracted at various times during infection suggest that many *wt* recombinants are formed within the first 5 min of growth (R. M. Benbow, Ph.D. thesis). Furthermore, UV irradiation of the parental phage before infection, which alters only the parental RF DNA, dramatically increases recombinant formation (28; R. M. Benbow et al., J. Mol. Biol., submitted for publication, 1973). Finally, the density shift experiments of R. M. Benbow (Ph.D. thesis) suggest that many φX174 recombinants are formed within the first 4 min of infection even when no selective conditions are used to block progeny RF DNA synthesis. Thus, it is reasonable to maintain that recombinant formation usually occurs between parental RF molecules early in infection.

It should be noted, however, that these data do not exclude the possibility that some progeny RF participate in the process, or that some late recombinant formation occurs in the major pathway. In fact, since we examined the genotype of the progeny phage, we may be more likely to observe the products of early events, since these a priori have an increased likelihood of being incorporated into phage particles.

Host recA gene product is required for most φX174 recombinant formation. A general

reduction in ϕX174 (J. Newbold and C. Hutchison, personal communication, 1966) and S13 (27) recombinant formation in a *rec*A host has been reported. Every marker-pair tested in ϕX174 has shown at least a two- to three-fold reduction (Table 5). In this paper we further establish (Table 5) that the reduction is proportionately greater within cistron A than in the rest of the genome.

We would like to emphasize that the major pathway for ϕX174 recombinant formation is defined in terms of the effect of the host *rec*A gene product (Tessman's primary mechanism; 27). In this context it is of interest that the *rec*A allele reduces the formation of bacterial recombinants by a factor of 10^3 (10), a considerably greater reduction than we have observed in ϕX174.

ϕX174 cistron products are not required for most ϕX174 recombinant formation. The crosses in Table 3 establish that the major pathway of recombinant formation is independent of the synthesis of functional products from cistrons A, B, D, E, F, G, and H. Neither C nor J could be tested directly for technical reasons. Cistron C, which does not affect early ϕX174 DNA replication (14), is a priori unlikely to alter the primary pathway. Cistron J, defined only by *am*6(J) (5), is also unlikely to alter ϕX174 recombinant formation because its DNA is replicated normally, and defective particles containing infective DNA are produced in nonpermissive host strains. With these two qualifications, we have shown that the major pathway does not depend on any known ϕX174 cistron product. That cistron A is not required was established previously (1).

Single recombination event usually generates only one recombinant genotype and one parental genotype. The data in Table 6 establish that, under the conditions employed, neither the reciprocal recombinant nor the second parental genotype is found in appreciable numbers in the bursts of single cells. Similar experiments in which no selection was employed confirm this finding (unpublished data) although occasional (<20%) mixed or reciprocal bursts, or both, were observed. Furthermore, the data of Denhardt and Silver (12) support the idea that the large number of recombinants seen in the observed bursts are typical of "normal" infections (i.e., that we are looking at normal recombination events). These conclusions agree with those for the unrelated filamentous bacteriophage f_1 (6–8).

An alternative, less efficient mechanism exists for the formation of ϕX174 recombinants in the absence of the host recA gene product. ϕX174 *wt* recombinants are formed in *rec*A cells at a frequency which is at least 100 times above the spontaneous reversion frequency (Tables 4 and 5). This suggests that a pathway which does not require the host *rec*A gene product is available for ϕX174 recombinant formation (27).

The existence of an independent pathway is suggested by the fact that UV light does not stimulate ϕX174 genetic recombination in *rec*A cells, although it is quite effective in rec^+ cells. This has been shown for ϕX174 (R. M. Benbow et al., J. Mol. Biol., submitted for publication, 1973) and earlier for S13 (28).

The ϕX174 cistron A product is required for maximal genetic recombination by this mechanism (Table 4). Baker et al. (1) suggest, reasonably, that the cistron A product is required for progeny RF DNA replication rather than for participation in the recombination process per se. This implies that the alternative pathway primarily involves progeny RF DNA molecules.

ϕX174 recombinant formation in the host double mutant *rec*A *rec*B strain is considerably less than that observed in a *rec*A host (Table 3). This implies that *rec*B may play some role in recombinant formation by the alternative mechanism. The decrease in recombinant formation in *rec*A *rec*B double mutants parallels the reduction observed when cistron A protein is not made during infection of a *rec*A host strain, but the relationship (if any) between the cistron A and *rec*B effects is unknown.

Significance of the major pathway of ϕX174 recombinant formation. The reason for carrying out these studies was to provide an experimental justification for the following assumptions upon which we relied heavily in our other research:

(i) The essential features of genetic recombination in bacteriophage ϕX174 may be investigated using conditions in which only parental RF DNA is present.

(ii) The major intermediates in ϕX174 recombination are present in rec^+ cells, but not in *rec*A cells, and their presence should not be affected by the ϕX174 mutants employed in any particular experiment.

These assumptions, coupled with the proportionality between the genetic and physical maps established previously (4, 5) are the genetic bases for our physical studies of ϕX174 recombinant formation (3; R. M. Benbow et al., J. Mol. Biol., submitted for publication, 1973 and R. M. Benbow, Ph.D. thesis).

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